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The field investigation for this project was conducted in Wilkinson Basin, Gulf of Maine (42°20'N; 69°47'W) from June 29 to July 6, 2000. Bioluminescent thin layers were located using the High-Intake-Defined Excitation-Bathyphotometer. Immediately following HIDEX-BP profiles the Johnson-Sea-Link submersible was launched with Dr. Katz's submersible holographic camera mounted on the upper work platform. Thin layers were located using redal-time sensor feedback fron intensified video recrodings of stimulated bioluminescence. Over 600 single and double exposure holograms (the latter for velocity measurements) were recorded in the upper 100m of the water column. Analysis is now underway to locate and identify particles within the holograms larger than 10 microns and to generate 3D vector arrays of instantaneous velocity distributions based on Holographic Particle Image Velocimetry

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# Investigation of Processes and Factors Regulating the Generation, Maintenance and Breakdown of Bioluminescent Thin Layers

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## LONG-TERM GOALS

My research objectives involve determining how low light phenomena, both bioluminescence and solar radiation below 200 meters, influence the distribution and behavior of marine organisms.

## OBJECTIVES

To investigate the phenomenon of bioluminescent thin layers discovered in Wilkinson Basin, Gulf of Maine (Widder et al., 1999). These thin layers (0.5 m) are composed of aggregations of the bioluminescent copepod *Metridia lucens* at density discontinuities in the water column. Three testable hypotheses were formulated to account for these aggregations: 1.) these copepods may enhance the repellent capacity of their bioluminescence by forming aggregations 2.) aggregation into thin layers may be an avoidance response to current shears or turbulent regions 3.) the copepods may have been orienting to some chemical cue or food resource such as marine snow that has been found at density discontinuities in the water column (MacIntyre et al., 1995).

## APPROACH

Using a computer algorithm, which we have recently developed for 3D reconstruction and statistical analysis of spatial point patterns of identified bioluminescent displays, we measured the median nearest neighbor distances of the bioluminescent copepods in these layers (Widder and Johnsen, 2000). Since this analysis indicated that the distance between individuals was nearly 10 times greater than the maximum perceptive distance of a copepod (Haury and Yamazaki 1995) we believe the first hypothesis, requiring as it does behavioral interaction between individuals, is the least probable of the three. To test the other two hypotheses and to further examine the factors that regulate the generation, maintenance and breakdown of these bioluminescent thin layers we conducted a field study in Wilkinson Basin in collaboration with Dr. Joe Katz of Johns Hopkins University. Thin layers were located using the High-Intake-Defined-Excitation-Bathyphotometer (Widder et al., 1993). Immediately following HIDEX-BP profiles the *Johnson-Sea-Link* submersible was launched with Dr. Katz's submersible holographic camera (Katz et al., 1999) mounted on the upper work platform (Figure 1). Thin layers were located using real-time sensor feedback from intensified video recordings of stimulated bioluminescence (Widder et al., 1989). Identification of organisms responsible for the bioluminescence was based on the spatial and temporal properties of the recorded displays. These displays were compared to our existing database of identified displays from this region (Widder et al., 1992; Widder, 1997).

Both single and double exposure holograms (the latter for velocity measurements) were collected during profiles through the layers and during transects in the layers.

## WORK COMPLETED

In order to mount the 1500 lb. holographic camera on the *Johnson-Sea-Link* submersible all other sampling gear including the robotic arm and the entire lower work platform had to be removed and a support frame had to be designed and fabricated that could support the weight of the camera in air. The electronics and control systems were mounted in front of the JSL and the optical system was mounted completely above it in order to minimize the effect of the submersible on the flow and plankton in the same volume (Fig. 1). During the field investigation in Wilkinson Basin, Gulf of Maine (42°20′N; 69°47′W) which took place June 29 – July 6, holograms were collected by locating a thin layer by its bioluminescence and then trimming the sub out just below the layer after first moving out from under any water disturbed by the submersible. A minimum amount of positive trim was then applied allowing the sub to drift up very slowly through the layer while a series of holograms were collected in rapid succession. The cylindrical sample volume for each hologram was 6.3 cm diameter and 28 cm long.

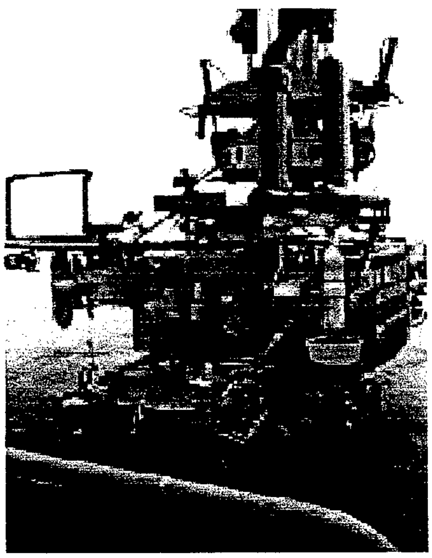

***Figure 1 Johnson-Sea-Link submersible with holocam mounted on upper work platform, port side. Sample volume is between upper most portion of blue fins.***

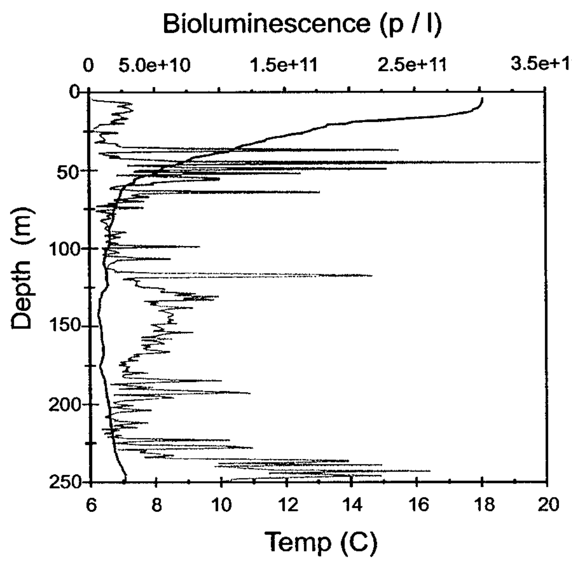


***Figure 2. HIDEX-BP profile in Wilkinson Basin showing bioluminescent thin layers.***

## RESULTS

A total of 47 HIDEX-BP casts were collected. Bioluminescent thin layers, similar to those recorded in the 1992 investigation, were present (Figure 2). Oblique bioluminescence video transects proved very effective in locating thin layers from the submersible. Over 600 single and double exposure holograms were recorded in the upper 100 m of the water column. These are the clearest and sharpest holograms ever recorded with this submersible system (Figure 3). Analysis is now underway to locate and identify particles within the holograms larger than 10 microns and to generate 3D vector arrays of instantaneous velocity distributions based on Holographic Particle Image Velocimetry (HPIV) (Tao et al., 1999). Plankton samples were pumped from depths where bioluminescence potential was high based on both HIDEX-BP and video transect data. These samples were completely dominated by copepods with three species present in significant numbers. These were *Metridia lucens* (almost entirely females), *Calanus finmarchicus* and *Oithona* spp. There were also very large numbers of exoskeletons (copepod molts) in the samples. Also present was the cydippid ctenophore *Euplokamis* sp. Although destroyed by the pump, these brilliantly luminescent ctenophores (Figure 4) were readily apparent in the video transects. The presence of this ctenophore in high abundance at relatively shallow depths (~40 m) was noteworthy because in past investigations in these waters it has always been found much deeper (> 200 m) (Widder et al., 1992; Widder 1997; Widder et al., 1999).

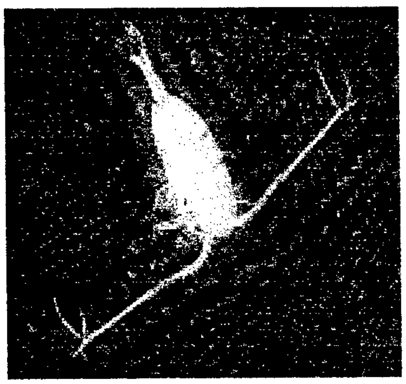

***Figure 3. Hologram of Calanus finmarchicus***

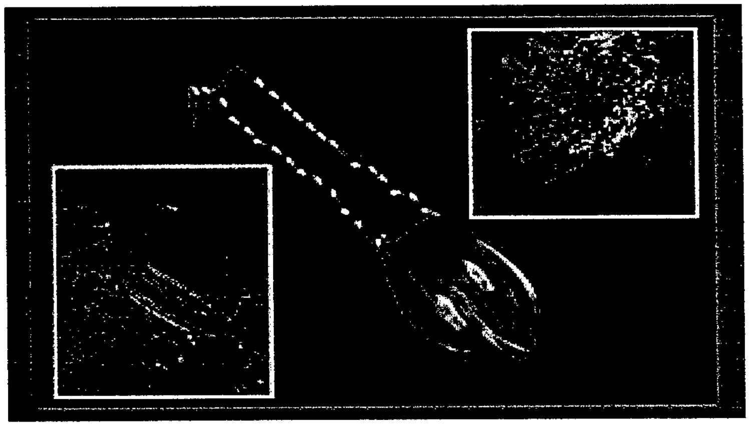

***Figure 4. The bioluminescent ctenophore Euplokamis sp. produces light both intrinsically (lower left) and extrinsically (upper right). The extrinsic bioluminescence consists of glowing particles that leave a persistent trail in the water.***

**IMPACT/APPLICATIONS**

From a strategic standpoint the existence of thin layers of intense bioluminescence could have a detrimental impact on covert naval operations and objectives. Because, these thin layers would not be detected by standard low flow (<1 l/s) bathyphotometer systems they would not be identified by realtime environmental nowcasts and forecasts. Therefore, while the average bioluminescence measured by a low-flow bathyphotometer system might indicate an average bioluminescence that was deemed acceptable for a nighttime stealth mission, that mission could be seriously compromised if a boat, SDV or swimmer encountered one of these bioluminescent hot zone.

From a scientific standpoint the existence of food-rich thin layers provides a possible explanation for why measured average *in situ* food concentrations appear to be inadequate for the daily metabolic requirements of marine grazers (Mullin and Brooks 1976; Dagg 1991; Gifford 1993; Batchelder and Williams 1995; Cowles and Fessenden 1995). Although the possibility has been raised that this disparity could be accounted for by the existence of food-rich micropatches (e.g. Batchelder and Williams 1995), this solution depends on how much energy grazers must expend to locate such patches (Haury et al. 1978). Locating food-rich thin layers, especially for vertical migrators, is less problematic since the search strategy can be reduced from three dimensions to one. A grazer moving up or down through the water column has a much higher probability of encountering food-rich micropatches if those patches are spread out into very thin layers. If such thin layers are in fact a common feature of the marine environment then their existence will have a profound impact on such critical aspects of population dynamics as species-habitat associations and encounter probabilities between predators and prey.

## TRANSITIONS

These data will be made readily available to NAVOCEANO and other Naval investigators as well as to the oceanographic community through publication in the open literature.

## RELATED PROJECTS

During the field investigation, tests were conducted on an expendable bathyphotometer system that is currently being developed with support from ONR (see report: "A Compact Bathyphotometer" by Fucile, Widder and Brink).

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